

Genomic Editing of the HIV-1 Coreceptor CCR5 in Adult Hematopoietic Stem and Progenitor Cells Using Zinc Finger Nucleases.

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Public Summary:

It is well known that infection with HIV-1 requires a protein called CCR5. Persons with a natural mutation in this gene (CCR5 Δ 32) are protected from HIV/AIDS. In an effort to control HIV-1 in an infected person, our goal is to develop a method to inactivate the CCR5 gene, which would possibly result in less spread of HIV-1 and potentially no need for continued anti-HIV-1 medications. Toward this goal, we used proteins that are called Zinc Finger Nucleases (ZFNs) that can disrupt the CCR5 gene in blood stem cells. With these ZFNs, we engineered a person's own blood stem cells (called autologous CD34⁺ cells) and then tested whether this method efficiently disrupted the CCR5 gene. We found that the method did efficiently modify the CCR5 gene; using special laboratory conditions, we achieved more than 25% CCR5 gene disruption in the stem cells. We then showed that these cells could still be transplanted and make an immune system in a mouse. These results establish a basis for a new approach exploiting a ZFN delivery system to modify the CCR5 gene of blood stem cells, and this method has potential as a future anti-HIV-1 treatment.

Scientific Abstract:

The HIV-1 coreceptor CCR5 is a validated target for HIV/AIDS therapy. The apparent elimination of HIV-1 in a patient treated with an allogeneic stem cell transplant homozygous for a naturally occurring CCR5 deletion mutation (CCR5 Δ 32/ Δ 32) supports the concept that a single dose of HIV-resistant hematopoietic stem cells can provide disease protection. Given the low frequency of naturally occurring CCR5 Δ 32/ Δ 32 donors, we reasoned that engineered autologous CD34⁺ hematopoietic stem/progenitor cells (HSPCs) could be used for AIDS therapy. We evaluated disruption of CCR5 gene expression in HSPCs isolated from granulocyte colony-stimulating factor (CSF)-mobilized adult blood using a recombinant adenoviral vector encoding a CCR5-specific pair of zinc finger nucleases (CCR5-ZFN). Our results demonstrate that CCR5-ZFN RNA and protein expression from the adenoviral vector is enhanced by pretreatment of HSPC with protein kinase C (PKC) activators resulting in >25% CCR5 gene disruption and that activation of the mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway is responsible for this activity. Importantly, using an optimized dose of PKC activator and adenoviral vector we could generate CCR5-modified HSPCs which engraft in a humanized mouse model (albeit at a reduced level) and support multilineage differentiation in vitro and in vivo. Together, these data establish the basis for improved approaches exploiting adenoviral vector delivery in the modification of HSPCs. *Molecular Therapy* (2013); doi:10.1038/mt.2013.65.

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